ACTA BIOLOGICA CRACOVIENSIA Series Botanica 42/1: 55–59, 2000

HETEROCHROMATIN IN *PLEUROZIUM SCHREBERI* (BRID.) MITT.

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Received September 21, 1999; revision accepted November 15, 1999

Differential staining of constitutive heterochromatin was used to investigate the karyotype of *Pleurozium schreberi* (n = 5), the object of Vaarama's (1954) classic work. C-banding demonstrated that the haploid karyotype is heterochromatin-rich. The total amount of heterochromatin is 44.1% of the karyotype length. Telomeric as well as intercalary bands were observed in particular chromosomes. Centromeric bands were recognized in one chromosome only. The banding pattern of chromosome A, the longest, largely resembles Vaarama's description. Heterochromatic bodies were also observed in interphase nuclei.

Key words: *Pleurozium schreberi*, heterochromatin, Giemsa C-banding, mosses.

INTRODUCTION

Pleurozium schreberi was the object of a seminal work by Vaarama (1954). His view of the evolutionary relationship between holocentric and monocentric chromosomes was presented in his study of mitosis and meiosis in this moss. Judging by the shapes of the arms of the largest bivalent during anaphase I, Vaarama, and later Anderson (1980), concluded that the large bivalent (designated the A-bivalent) has more than one active kinetic element. In Vaarama's opinion, *P. schreberi* represents an intermediate condition between holocentric and monocentric chromosomes. In mitosis, however, the A chromosome of this moss is clearly monocentric, as shown in Vaarama's figures. Vaarama concluded by calling them chromosomes with semilocalized centromeres. A schematic figure in Vaarama's paper (1954 p. 14, Fig. 20) illustrates his view of the likely structure of the A-bivalent at metaphase I, with one arm of chromosome A almost totally heterochromatic (named the h-arm). He thought this chromosome corresponded to the big heteropycnotic body observed in interphase nuclei. The second arm was described as euchromatic (e-arm) with four heterochromatic blocks. Vaarama assumed that the A-bivalent was composed of four approximately equal chromosome blocks. The primary constriction separates the chromosome arms (named the two primary blocks), and each arm was thought to be divided into two secondary blocks by secondary constrictions. Vaarama concluded that this compound structural character was caused by fusion of whole chromosomes. He based this hypothesis on observations of chromosomes stained in aceto-orcein. No information on the amount of heterochromatin in chromosomes of *P. schreberi* was given. Anderson (1980) stated that such information would be a useful contribution.

To identify constitutive heterochromatin in the chromosomes of P. schreberi we undertook karyotype analysis using a C-banding method. Differential staining has been little used in mosses. Only six species (*Atrichum undulatum, A. crispum, Dicranum tauricum, Gymnostomum aeruginosum, Plagiomnium ellipticum, and Rhizomnium pseudopunctatum*) are known to have been investigated using this technique (Newton, 1977, 1979, 1983a, 1984a).

MATERIALS AND METHODS

Living material was collected in Kostrze near Cracow in 1995–96. The plants were harvested random-

PL ISSN 0001-5296

ly from a 30 × 30 m population area. Voucher specimens are deposited in the Department of Plant Cytology and Embryology of the Jagiellonian University in Cracow. The plants were grown in large glass containers with glass covers, on soil taken from their natural habitat. The containers were kept in a growth chamber with a 13 h photoperiod, at 15°C day/12°C night and 70–80% humidity. Material for cytology was taken throughout the period of cultivation. Gametophytic apices were excised randomly from plants. A total of ~ 700 gametophytic apices were examined.

Dissected shoot apices were treated in a saturated solution of alfabromonaphthalene for 24 h at 4°C, then fixed in 1:3 acetic alcohol (1 part glacial acetic acid, 3 parts absolute ethanol) for 24 h at 4°C and stained immediately.

Material was prepared for Giemsa C-banding of chromosomes according to Schwarzacher et al. (1980). Analyses of karyotypes were based on squashed material mounted in Euparal. Chromosome measurements were made from drawings taken from photo slides projected on a screen (× 20000). Chromosome nomenclature is that of Levan et al. (1964). For calculations and idiogram drawings, the Mr Karyo ver. 3.10 (by Tokarski and Joachimiak) program was used. Microphotographs were taken using a Nikon Labophot-2 microscope with an FX-35DX attachment, on Agfa APX 25 film and Kodak EPT 160T color slides.

RESULTS AND DISCUSSION

Pleurozium schreberi possesses one of the lowest chromosome numbers known in mosses, n = 5 (Fritsch, 1991). Vaarama (1954) suggested that the haploid complement was derived from an 11-unit set by end-to-end fusion of 10 chromosomes. In our investigated material, the number n = 5 was invariably established, confirming the karyological uniformity of this species. It should be mentioned, however, that in a previous paper concerning karyotype variability in *P. schreberi*, the number n = 6 was found in two gametophytes from the same population (Kuta et al., 1998).

A total of 120 slides was examined and the results were based on measurements of 12 prometaphases and metaphases. In the analyzed material the C-banding pattern in all chromosomes was well defined in only one prometaphase plate (k/4). A heterochromatin idiogram (Fig. 1) was constructed on the basis of this plate, and then this plate was chosen for presentation (Figs. 2, 3). In the remaining 11 plates the heterochromatic bands were poorly defined; generally, whole chromosomes were stained when C-banding technique was applied, due to the very condensed state of the metaphase chromosomes (Fig. 4, Tab. 1). Total chromosome length in plate k/4 was 30.75 μm, whereas the total lengths of the remaining 11 plates ranged from 13.5 to 23.4 μm (mean total length 18.7 μm), indicating that their chromosomes were markedly shorter (Tab. 1). C-positive bands were observed in relatively uncondensed prometaphase chromosomes. Heterochromatic bands probably fused together in condensed metaphase chromosomes, especially in heterochromatin-rich karyotypes. Similar observations have been reported in flowering plants and animals with monocentric and holocentric chromosomes (e.g., see: Ray and Venketeswaran, 1978; Collet and Westerman, 1984; Manicardi et al., 1991; Manicardi et al., 1996).

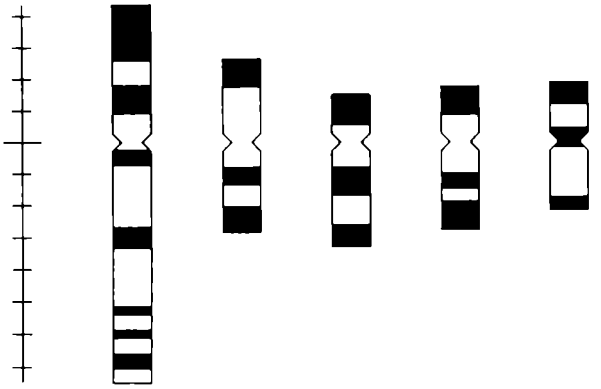


Fig. 1. C-band idiogram of *Pleurozium schreberi* (n = 5).

We also observed several other plates in which bands were clearly seen in particular chromosomes; for example, telomeric bands were visible in almost all chromosomes, whereas intercalary bands were visible only in less condensed ones. These plates were not measured, however, because the centromeres were not recognizable [a well known phenomenon in bryophytes (see: Przywara and Kuta, 1995)]. It should be noted that heterochromatin bands were not visible in fresh preparations (Fig. 2). A banding pattern appeared on slides after several weeks (Fig. 3), as observed in angiosperm chromosomes stained in Giemsa for C-bands.

In the haploid set of *P. schreberi* the largest chromosome (chromosome A) is nearly twice the

TABLE 1. Chromosome length (μm) in 12 selected plates stained using Giemsa C-banding

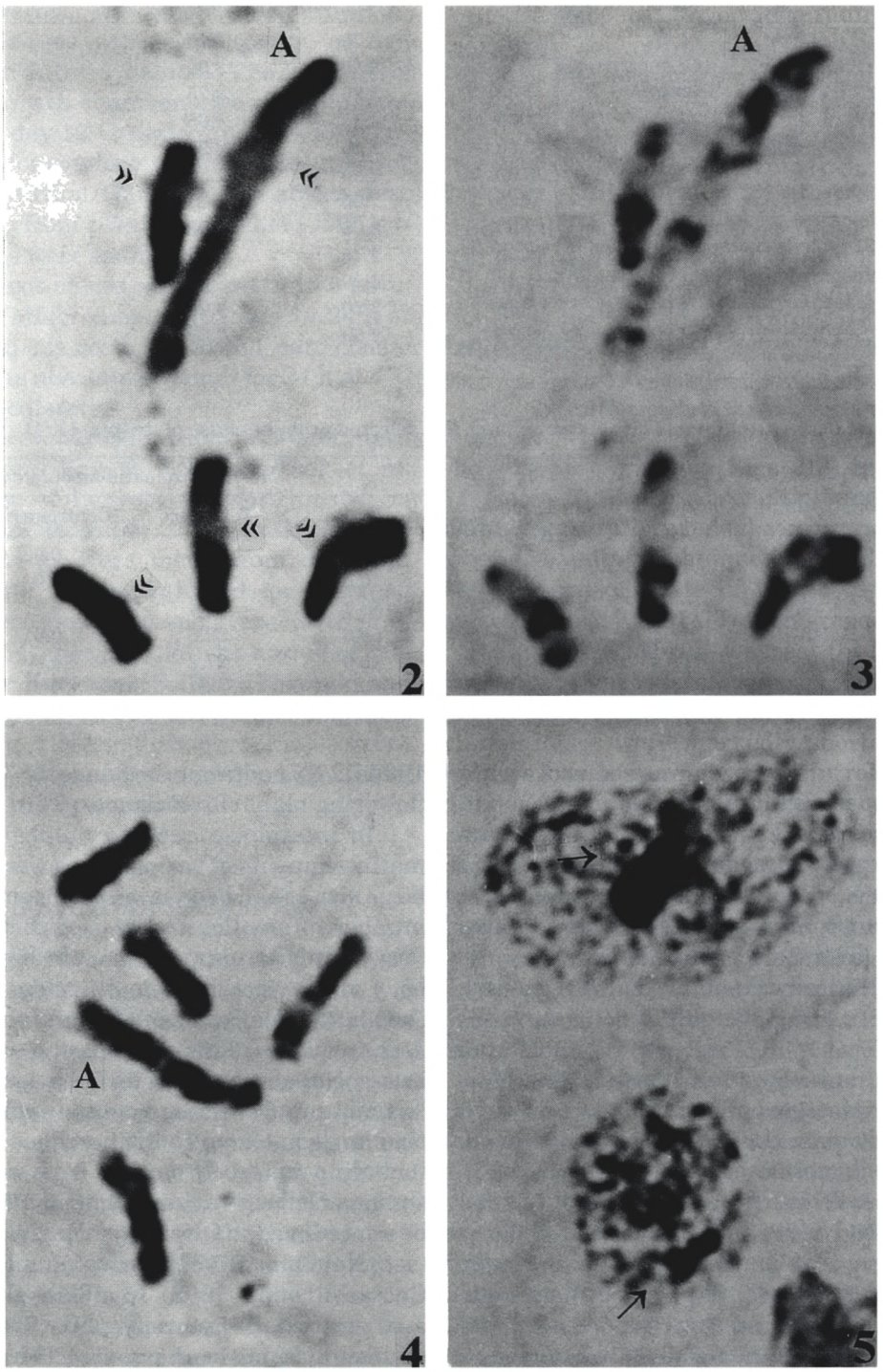
Plate	Chromosome					Total chromosome length
	1st	2nd	3rd	4th	5th	
k/1	6.55	4.70	4.35	3.70	3.70	23.00
k/2	6.65	4.80	4.30	3.30	3.00	22.05
k/3	6.60	4.85	4.40	3.80	3.75	23.40
k/4	11.85	5.50	4.80	4.45	4.05	30.65
k/5	4.05	3.00	2.60	2.20	2.20	14.05
k/6	5.10	2.75	3.00	2.50	2.50	15.85
k/7	4.80	2.80	2.25	2.60	2.45	14.90
k/8	4.15	2.80	2.50	2.05	2.00	13.50
k/9	4.95	3.35	3.20	3.15	2.65	17.30
k/10	6.15	4.90	4.45	4.20	3.65	23.35
k/11	6.65	3.60	3.45	3.20	3.00	19.90
k/12	7.70	3.00	2.75	2.60	2.55	18.60

TABLE 2. C-banding pattern and heterochromatin amount in a selected prometaphase plate (plate k/4)

Chromosome	Total length (μm)	Arm ratio	No. of C-bands	Heterochromatin amount (%)			
				Total	Telomeric	Centromeric	Intercalary
1st	11.85	1.72	7	40.1	14.8	0.0	25.3
2nd	5.50	1.08	3	40.9	30.9	0.0	10.0
3rd	4.80	2.20	3	53.1	34.4	0.0	18.7
4th	4.45	1.53	3	50.6	39.6	0.0	11.0
5th	4.05	1.13	3	42.0	27.2	14.8	0.0

length of the next largest chromosome (Tab. 1). Chromosome A is metacentric or submetacentric, showing polymorphism regarding centromere position and the presence of secondary constrictions (Kuta et al., 1998). The C-banded karyotype of *P. schreberi* is heterochromatin-rich (44.1% of karyotype length) (Tab. 2). All chromosomes show telomeric blocks of heterochromatin: chromosomes 2, 3, 4 and 5 on both arms and chromosome A on the shorter arm (Figs. 1, 3). Intercalary bands were identified in chromosomes 1–4. Centromeric heterochromatin was recognized in chromosome 5 only. Seven heterochromatic bands differing in size were observed in the longest A chromosome (Figs. 1, 3). The shorter arm of this chromosome is highly heterochromatic (61% of its length), with two large bands. The longer arm is mostly euchromatic, with five narrow bands representing 28% of its length. The larger intercalary band observed on the longer arm could be an effect of the fusion of two bands; on the other hand, three small blocks at the end of this arm may have fused together to form the one large telomeric block indicated on Vaarama's schematic figure. Interestingly, in all published C-banded idiograms of mosses (Newton, 1977, 1979, 1983a, 1984a), no centromeric bands or dots, typical of flowering plant chromosomes, were recognized.

In interphase nuclei a number of heterochromatin segments of various sizes were observed. The large size of one body is very conspicuous; the exact number of smaller bodies is difficult to establish (Fig. 5). In Vaarama's opinion the big heteropycnotic body visible in the haploid nucleus corresponds to the almost totally heterochromatic short arm of the A chromosome. This chromosome was suggested to be associated with sex determination, as *P. schreberi* is a dioecious species. Another explanation is that this large heteropycnotic body is a collective body including heterochromatin from several chromosomes. Collective heterochromatic bodies have been observed in interphase nuclei of other plants (see: Newton, 1977; Patankar and Ranjekar, 1988; Ceccarelli et al., 1998). In mosses the number, size and position of heteropycnotic bodies have been studied by some workers since the classic paper of Heitz (1928) (for review: Smith, 1978; Newton, 1983b; 1984b). Their presence was often correlated with H and h chromosomes (depending on size) identified as sex-specific chromosomes (e.g. Tatuno, 1941).



Figs. 2–4. C-band chromosomes of *Pleurozium schreberi* (n = 5). **Fig. 2**. Prometaphase chromosomes photographed immediately after staining; banding pattern not visible; position of centromeres indicated with double arrowheads. **Fig. 3**. The same chromosomes photographed 8 weeks after staining; banding pattern clearly visible in all chromosomes. **Fig. 4**. Condensed metaphase chromosomes; banding pattern slightly visible. A – largest chromosome of the complement. All figures × 4400. **Fig. 5**. Interphase nuclei; large heterochromatic bodies indicated with arrows. × 4400.

The karyotype of *P. schreberi* is heterochromatin-rich. The data indicate that the pattern of constitutive heterochromatin distribution in chromosome A approximately corresponds to Vaarama's results based on classically stained meiotic and mitotic chromosomes.

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